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PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 16 August 1999 (16.08.99)	
International application No. PCT/EP98/07313	**Applicant's or agent's file reference** B 3077 PCT
International filing date (day/month/year) 16 November 1998 (16.11.98)	**Priority date** (day/month/year) 17 November 1997 (17.11.97)
Applicant KUFER, Peter et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

16 June 1999 (16.06.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

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Authorized officer

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Q2

REPLACED BY ART. 34 AMDT.

17

REC'D 18 FEB 2000
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PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
B 3077 PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP98/07313	16/11/1998	17/11/1997
International Patent Classification (IPC) or national classification and IPC C12N15/10		
Applicant KUFER, Peter et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
16/06/1999	14.02.00
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Moonen, P Telephone No. +49 89 2399 8538

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP98/07313

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-54	as originally filed			

Claims, No.:

1-29	as received on	07/01/2000	with letter of	07/01/2000

Drawings, sheets:

1/48-48/48	as originally filed

Drawings, No.:

1-12	as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 1-20, 22-29
	No:	Claims 21
Inventive step (IS)	Yes:	Claims 9, 12-18 and 22-29
	No:	Claims 1-8, 10-11 and 19-21
Industrial applicability (IA)	Yes:	Claims 1-29
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1: PNAS **92** (1995) 7021-5
D2: EP-A-0 610 046
D3: Nature Biotechnology **14** (1996) 1149-1154

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The present application is directed to the selection of recombinant bi- or multivalent single-chain (sc) **fusion** proteins. A selected sc-protein may comprise a binding site domain binding strongly to a predetermined epitope and C-terminal to **another,** additional domain (this additional domain being undefined in claim 1); in other words, it is considered that the present invention does not necessarily only relate to the identification of **single** binding sites that can subsequently be joined as multicompatible modules (claim 1 refers to the display of the fusion protein containing at least two domains). The affinity of binding to the epitope is not influenced by the position of the binding site domain in the recombinant fusion protein.

The selection for binding is not a novel concept: **D3** has referred to the selection for binding in relation to the obtaining of more stable diabodies applying phage display libraries. An efficient selection procedure to enrich for antigen-binding diabodies is applied (see Table 1). The first full paragraph on page 1153 reads as follows, following on a discussion on the affinity of the D1.3 lysozyme binding: In constructing an individual bispecific molecule from two antibodies, it will be necessary to carefully measure the effect of one binding site on its partner. However, by constructing and selecting from a library, potentially unfavourable combinations could be avoided. It may even be possible to select from large collections of bispecific molecules those diabodies that exhibit an allosteric effect.

No prior art document has specifically referred to the C-terminal positioning in a recombinant bi- or multivalent polypeptide; however, in view of the above teaching of D3 (considered to be the closest prior art document) with the general

knowledge about the generation of sc proteins and the possibility to screen for the desired binding specificities (see also **WO 94/05781** page 5 lines 26-30) it is considered to have been obvious to measure the effect of C-terminal positioning of a binding site domain.

In the construction of a coding sequence for a bi-specific antibody the skilled person has two options concerning the order of the domain coding sequences; in view of the teaching of D3 it is considered to have been obvious to consider the generation of both constructs.

Therefore, the present application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of **claim 1** does not involve an inventive step (Rule 65(1)(2) PCT).

2. With respect to the depending **claims 2-8, 10-11 and 19-20** it is considered that the additional features of these claims are obvious to the skilled person as they appear to be based on known aspects for the construction of recombinant vectors coding for fusion proteins containing at least two domains.

3. With respect to present **claim 9** and depending **claims 12-18** an inventive step is acknowledged for the additional domain specified as the N2-domain of the gene III product of filamentous phage; the advantagous effect of the presence of this domain N-terminal of the binding domain was not obvious to the skilled person (Article 33(3) PCT).

4. With respect to the kit-claim 21 referring back to the fusion proteins of claim 1 it is considered that this claim is non-unitarily linked to the method-claims 1-8, as the subject-matter of claim 21 does not necessarily depend on the method of claim 1. The subject-matter of said claim overlaps with the products obtainable from methods already available in the prior art (see e.g. **D1,** the C-terminal domain of Flag is a binding site domain, and see also **D3**, the bispecific fusion proteins). Thus, it is considered that **claim 21** lacks novelty, contrary to the requirements of Article 33(2) PCT.

5. **Claims 22- 29** relate to specified CDR amino acid sequences generated by the method of the invention; the specified sequences are considered to involve an inventive step.

Re Item VII

Certain defects in the international application

6. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D3 is not mentioned in the description, nor is this document identified therein.

Re Item VIII

Certain observations on the international application

7. In conjunction with the above observation with respect to the lack of unity of invention, it is noted that Article 6 of the PCT requires that all independent claims contain the essential technical feature(s) of the invention (see also Rule 6.3(b) PCT).

   This special technical feature of the invention is considered to be the addtional domain referred to in present claim 9.

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CLAIMS

1. A method of identifying a binding site domain having the capacity of binding to a predetermined epitope when positioned C-terminal of at least one further domain in a recombinant bi- or multivalent polypeptide comprising the steps of

   (a) testing a panel of binding site domains displayed on the surface of a biological display system as part of a fusion protein for binding to a predetermined epitope, wherein said fusion protein comprises an additional domain positioned N-terminal of said binding site domain and an amino acid sequence that mediates anchoring of the fusion protein to the surface of said display system; and

   (b) identifying a binding site domain that binds to said predetermined epitope.

2. The method of claim 1, wherein said binding site domain and said additional domain are linked by a polypeptide linker disposed between said binding site and said additional domain, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids and connects the N-terminal end of said binding site domain and the C-terminal end of said additional domain.

3. The method of claim 1 or 2, wherein said binding site domain is a pair of V_H-V_L, V_H-V_H or V_L-V_L domains.

4. The method of any one of claims 1 to 3 wherein said display system is a filamentous phage produced by bacteria transfected therewith, a baculovirus expression system, a ribosome based expression system, a bacteriophage lambda display system or a bacterial surface expression system.

5. The method of claim 4 comprising, prior to step (a), the further step of

   (a") transfecting bacteria with recombinant vectors encoding said fusion proteins.

6. The method of any one of claims 1 to 5 comprising, prior to step (a"), the further step of

(a') cloning a panel of nucleic acid molecules encoding said binding site domains into a vector.

7. The method of claim 6, wherein said panel of nucleic acid molecules is derived from immune competent cells of a mammal, fish or bird.

8. The method of any one of claims 1 to 7, wherein said additional domain comprises at least 9 amino acids.

9. The method of claim 8, wherein said additional domain is or is derived from the N2-domain of the gene III product of filamentous phage.

10. The method of any one of claims 1 to 9, wherein said sequence that mediates said anchoring is or is derived from the C-terminal CT-domain of the gene III product of filamentous phage.

11. The method of any one of claims 1 to 8, wherein said bi- or multivalent polypeptide is a bi- or multifunctional polypeptide.

12. The method of claim 9, wherein said at least one further domain comprises polypeptide selected from the group consisting of effector proteins having a conformation suitable for biological activity, amino acid sequences capable of sequestering an ion, and amino acid sequences capable of selective binding to a solid support.

13. The method of claim 12 wherein said effector protein is an enzyme, toxin, receptor, binding site, biosynthetic antibody binding site, growth factor, cell-differentiation factor, lymphokine, cytokine, hormone, a remotely detectable moiety, or anti-metabolite.

14. The method of claim 12 wherein said sequence capable of sequestering an ion is calmodulin, methallothionein, a fragment thereof, or an amino acid sequence rich in at least one of glutamic acid, aspartic acid, lysine, and arginine.

15. The method of claim 12 wherein said polypeptide sequence capable of selective binding to a solid support is a positively or negatively charged amino acid sequence, a cysteine-containing amino acid sequence, streptavidin, or a fragment of Staphylococcus protein A.

16. The method of claim 13, wherein said receptor is a co-stimulatory surface molecule important for T-cell activation or comprises an epitope binding site or a hormone binding site.

17. The method of claim 16, wherein said co-stimulatory surface molecule is CD80 (B7-1), CD86 (B7-2), CD58 (LFA-3) or CD54 (ICAM-1).

18. The method of claim 17, wherein said epitope binding site is embedded in a pair of V_H-V_L, V_H-V_H or V_L-V_L domains.

19. The method of any one of claims 3 to 18, wherein said pair of domains are connected by a flexible linker, preferably by a polypeptide linker disposed between said domains, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of one of said domains and the N-terminal end of the other of said domains when said fusion protein assumes a conformation suitable for binding when disposed in aqueous solution.

20. The method of any one of claims 1 to 19, wherein the identification of said binding site domain comprises the steps of

(b') removing said amino acid sequence that mediates anchoring of the fusion protein to the surface of a phage from said fusion protein;

(b") periplasmatically expressing the nucleic acid molecules encoding the remainder of said fusion protein in bacteria; and

(b"') verifying whether said binding site domain binds to said predetermined epitope.

21. A recombinant vector as defined in anyone of claims 1 to 20.

22. A host cell harboring and capable of suppressing the recombinant vector of claim 21.

23. Kit comprising

(a) a recombinant vector of claim 21 or a panel of recombinant vectors encoding a panel of fusion proteins as defined in any one of claims 1 to 20; and/or

(b) a host cell of claim 22 or a bacterial library transfected with a panel of vectors as defined in (a).

24. A binding site domain or fusion protein obtainable by the method of any one of claims 1 to 20.

25. The binding site domain or fusion proteins of claim 24, wherein said binding site domain comprises at least one complementarity determining region (CDR) of the scFv fragment shown in any one of figures 6.3 to 6.10 and 7.

26. A polypeptide or an antibody comprising at least one binding site domain or fusion protein of claim 24 or 25.

27. The polypeptide or antibody of claim 26 having the amino acid sequence as depicted in any one of figures 6.3 to 6.10 and 7.

28. Polynucleotides which upon expression encode the polypeptide or antibody of claim 26 or 27.

29. A cell transfected with a polynucleotide of claim 28.

30. A process for the preparation of a polypeptide or antibody of claim 26 or 27 comprisind cultivating a cell of claim 29 under conditions suitable for the expression of the polypeptide and isolating the polypeptide from the cell culture medium.

31. A pharmaceutical composition containing a polypeptide or antibody of claim 26 or 27 and optionally a pharmaceutically acceptable carrier.

32. A diagnostic composition comprising the polypeptide or antibody of claim 26 or 27 and optionally suitable means for detection.

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PATENT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
B 3077 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/EP 98/ 07313	16/11/1998	17/11/1997
Applicant		
KUFER, Peter et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[X] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

METHOD OF IDENTIFYING BINDING SITE DOMAINS THAT RETAIN THE CAPACITY OF BINDING TO AN EPITOP

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[ ] as suggested by the applicant.

[X] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

INTERNATIONAL SEARCH REPORT

[redacted]ational Application No
[redacted]/EP 98/07313

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/10 C12N15/13 C12N15/62 C12N5/10 C12N1/21
C07K14/705 C07K16/30 A61K39/395 G01N33/577

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MACK M ET AL: "A SMALL BISPECIFIC ANTIBODY CONSTRUCT EXPRESSED AS A FUNCTIONAL SINGLE-CHAIN MOLECULE WITH HIGH TUMOR CELL CYTOTOXICITY" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 92, no. 15, 18 July 1995, pages 7021-7025, XP000566333 cited in the application see the whole document ---	24,26, 28-32
X	EP 0 610 046 A (SQUIBB BRISTOL MYERS CO) 10 August 1994 see the whole document, esp. pp.5-8 --- -/--	24,26, 28-32

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 April 1999	28/04/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 05781 A (SCRIPPS RESEARCH INST ;LIGHT JAMES PAUL II (US); LERNER RICHARD A) 17 March 1994 see the whole document	1-32
A	WO 92 18619 A (SCRIPPS RESEARCH INST) 29 October 1992 see the whole document	1-32
A	MCGUINNESS, BRIAN T. ET AL: "Phage diabody repertoires for selection of large numbers of bispecific antibody fragments" NAT. BIOTECHNOL. (1996), 14(9), 1149-1154 CODEN: NABIF9;ISSN: 1087-0156, XP002100039 see the whole document	1-32
A	MACK M ET AL: "Biologic properties of a bispecific single-chain antibody directed agains 17-1A (EpCAM) and CD3: tumor cell-dependent T cell stimulation and cytotoxic activity." JOURNAL OF IMMUNOLOGY, (1997 APR 15) 158 (8) 3965-70. JOURNAL CODE: IFB. ISSN: 0022-1767., XP002100040 United States see the whole document	1-32
A	CLACKSON T ET AL: "IN VITRO SELECTION FROM PROTEIN AND PEPTIDE LIBRARIES" TIBTECH, vol. 12, May 1994, pages 173-184,. XP000652419 see esp. pp.174-179 1.col.	1-32
A	HAYDEN, MARTHA S. ET AL: "Antibody engineering" CURR. OPIN. IMMUNOL. (1997), 9(2), 201-212 CODEN: COPIEL;ISSN: 0952-7915, XP002100041 see esp. p.206 r.col. ff.	1-32

INTERNATIONAL SEARCH REPORT

[redacted]tion on patent family members

[redacted]national Application No
PCT/EP 98/07313

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0610046 A	10-08-1994	US 5637481 A	10-06-1997
		CA 2114353 A	02-08-1994
		JP 6319548 A	22-11-1997
WO 9405781 A	17-03-1994	AU 685753 B	29-01-1998
		AU 4848593 A	29-03-1994
		CA 2143104 A	17-03-1994
		EP 0663953 A	26-07-1995
		JP 8502645 T	26-03-1996
		US 5770356 A	23-06-1998
WO 9218619 A	29-10-1992	AU 662148 B	24-08-1995
		AU 1785692 A	17-11-1992
		CA 2108147 A	11-10-1992
		EP 0580737 A	02-02-1994
		FI 934422 A	08-12-1993
		JP 6506836 T	04-08-1994
		NO 933610 A	10-12-1993
		PT 100379 A,B	31-08-1993
		US 5658727 A	19-08-1997
		US 5759817 A	02-06-1998
		US 5667988 A	16-09-1997